



US009410173B2

(12) **United States Patent**
Betts et al.(10) **Patent No.:** **US 9,410,173 B2**
(45) **Date of Patent:** **Aug. 9, 2016**(54) **TEMPLATE SWITCH-BASED METHODS FOR PRODUCING A PRODUCT NUCLEIC ACID**(71) Applicant: **Clontech Laboratories, Inc.**, Mountain View, CA (US)(72) Inventors: **Craig Betts**, Mountain View, CA (US); **Andrew Alan Farmer**, Mountain View, CA (US)(73) Assignee: **Clontech Laboratories, Inc.**, Mountain View, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/058,038**(22) Filed: **Oct. 18, 2013**(65) **Prior Publication Data**

US 2014/0113332 A1 Apr. 24, 2014

Related U.S. Application Data

(60) Provisional application No. 61/717,973, filed on Oct. 24, 2012.

(51) **Int. Cl.**
C12P 19/34 (2006.01)
C12N 15/10 (2006.01)(52) **U.S. Cl.**
CPC **C12P 19/34** (2013.01); **C12N 15/1096** (2013.01); **C12Q 2521/107** (2013.01); **C12Q 2525/191** (2013.01)(58) **Field of Classification Search**
CPC C12Q 1/6806; C12Q 1/6853; C12Q 2521/107; C12Q 2525/155; C12Q 2525/161; C12Q 2525/173; C12Q 2525/191
See application file for complete search history.(56) **References Cited****U.S. PATENT DOCUMENTS**

5,962,271	A	10/1999	Chenchik et al.
5,962,272	A	10/1999	Chenchik et al.
6,251,639	B1 *	6/2001	Kurn C12Q 1/6844 435/6.1
6,406,890	B1	6/2002	Mueller
2006/0099589	A1	5/2006	Pedersen et al.
2008/0145844	A1	6/2008	Barsova et al.
2012/0283106	A1	11/2012	Wang et al.

FOREIGN PATENT DOCUMENTS

DE	196 01 385	A1	7/1997
EP	1 369 480	A1	12/2003
KR	2002-0036665	A	5/2002
WO	97/24455	A2	1/1996

WO	WO 01/09310	A1	2/2001
WO	WO 02-00938	A2	1/2002
WO	02/68629	A2	9/2002
WO	WO 2005/019452	A1	3/2005
WO	2012/042374	A2	4/2012
WO	WO 2012/116146	A1	8/2012
WO	2014/066179	A1	5/2014
WO	WO 2015/173402	A1	11/2015

OTHER PUBLICATIONS

Kaptein et al. BMC Genomics, 2010, 11: 413.*

Kurzynska-Kokorniak, et al. "DNA-directed DNA Polymerase and Strand Displacement Activity of the Reverse Transcriptase Encoded by the R2 Retrotransposon," Journal of Molecular Biology, vol. 374, No. 2, pp. 322-333 (2007).

Oz-Gleenberg et al., "Reverse transcriptases can clamp together nucleic acids strands with two complementary bases at their 3'-termini for initiating DNA synthesis," Nucleic Acids Research, vol. 39, No. 3, pp. 1042-1053, (2011).

Oz-Gleenberg et al., "Substrate variations that affect the nucleic acid clamp activity of reverse transcriptases," FEBS Journal, vol. 279, No. 10, pp. 1894-1903 (2012).

Islam et al. "Highly multiplexed and strand-specific single-cell RNA 5' end sequencing", Nature Protocols 7 (2012), 813-828.

Kivioja et al. "Counting absolute numbers of molecules using unique molecular identifiers", Nature Methods 9 (2012), 72-74.

Levin et al. "Comprehensive comparative analysis of strand-specific RNA sequencing methods", Nature Methods 7 (2010), 709-715.

Matz et al. "Amplification of cDNA ends based on template-switching effect and step-out PCR," Nucleic Acids Research, 1999, vol. 27, No. 6, 1558-1560.

Zhuang et al. "Structural bias in T4 RNA ligase-mediated 3'-adaptor ligation", Nucleic Acids Research 40(7): e54 (2012), 14 pages.

Schramm et al. "A simple and reliable 5'-RACE approach", Nucleic Acids Research, 2000, vol. 28, No. 22, e96, 4 pages.

Turchinovich et al. "Capture and Amplification by Tailing and Switching (CATS): An ultrasensitive ligation-independent method for generation of DNA libraries for deep sequencing from pictogram amounts of DNA and RNA", RNA Biology, vol. 11, No. 7, pp. 817-828 (Jul. 2014).

* cited by examiner

Primary Examiner — Angela M Bertagna(74) *Attorney, Agent, or Firm* — Bret E. Field; Bozicevic, Field & Francis LLP(57) **ABSTRACT**

Provided are methods of producing a product nucleic acid. The methods include combining a template deoxyribonucleic acid (DNA), a polymerase, a template switch oligonucleotide, and dNTPs into a reaction mixture. The components are combined into the reaction mixture under conditions sufficient to produce a product nucleic acid that includes the template DNA and the template switch oligonucleotide each hybridized to adjacent regions of a single product nucleic acid that includes a region polymerized from the dNTPs by the polymerase. Aspects of the invention further include compositions and kits.

15 Claims, 1 Drawing Sheet